Claims 1 to 24 and 25 to 29 stand rejected under 35 USC 112, second paragraph, for being indefinite and failing to particularly point out and distinctly claim the subject matter which the applicants regard as the invention. Applicants have deleted claims 20 to 23 and claim 25 and amended remaining claims to include anticedent definitions for clarity of phrases and to place applicable claims in proper Markush format.

The claims are now directed to the generation of transgenic *alkaloid producing poppy* plants. Further to this, Applicants note the phrase "exogenous genetic material" has been amended to read *exogenous nucleic acid* for conferring a selected property on the transgenic plant of the invention. Support for this is found in the specification at for instance, page 5, lines 8 to 25, which state that the genetic material may be DNA or RNA and may encode a gene or a fragment of a gene, or it may represent antisense nucleotide sequences genes.

In addition, the claims have been amended to make it clear where applicable, that by the phrase "plant material" is meant the plant material from which the transgenic plant is generated. In this regard, and with reference to claim 1 as amended, Applicants submit the claim specifically requires in step (3) that the transgenic plant be generated from the plant material. Accordingly, the claims inherently require that the plant material must be suitable plant material from which the transgenic plant is capable of being generated. Suitable plant material includes for instance seed explant, seedling explant, type I callus, type II callus and somatic embryogenic callus as stated in the specification at page 4, lines 19 to 23. Hence, the plant material may be selected from a range of possible plant materials and it is respectfully submitted that amending the claims to recite the plant material as being a plant or explant as suggested by the Examiner would be unduly limiting given the disclosure in the specification provided by the Applicants.

Further support for the amendments is found in the specification at, for instance, page 4, lines 16 to 18, which state that preferably the transgenic plant of the invention is an alkaloid producing poppy plant and in particular, an *Eschscholtzia* or *Papaver* species, and desirably, a *Papaver somniferum*. Reference is also made to the Examples which teach the generation of a transgenic *Papaver somniferum* in accordance with the methods of the present invention.

In addition, attention is drawn to page 3, lines 9 to 15 which state that the Applicants have found that there is an unexpected and rapid rise in pH of the culture medium when poppy cells are cultured that is at least in part responsible for the lack of success in regenerating poppy plants and recovering transgenic poppy plants in the past. The disclosure

at page 4, lines 24 to 27, also teaches that the buffering agent can be used to prevent or delay the rapid rise in pH.

Claims 21 to 23 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is mostly connected, to make and/or use the invention. Applicants have cancelled claims 21 to 23 without prejudice in order to advance the progress of this application.

Claims 1 to 5, 7-26, 28 and 29 are rejected under 35 of U.S.C. 112, first paragraph, on the basis that the specification, while being enabling for a method of producing a transgenic *Papaver somniferum* utilising a buffered medium, does not reasonably provide enablement for a method of producing any transgenic plant, or *Papaver* or *Eschscholtzia* species.

The claims as amended are directed to methods of producing transgenic alkaloid producing poppy plants, the resulting transgenic plants and the transformed plant material produced in methods of the invention. As discussed above, and as stated at page 3 lines 9 to 15, the invention is based on the unexpected finding that there is a rapid rise in the pH of the culture medium during cultivation of poppy cells and further, this rise in pH is observed around poppy cultures including initial explants (eg. seedling hypocotyls), undifferentiated callus, and callus regenerated via somatic embryos or shoots. As further stated at page 7, lines 14 to 18 of the specification, this very rapid and substantial rise in the immediate area around, for instance, type II callus has been identified by the present inventors as a major cause of poor growth and difficulty in producing transgenic poppies. Having recognised the problem, the Applicants found that by transforming and/or culturing transformed poppy cells in the presence of a buffering agent which resists significant changes in pH, a substantial improvement in obtaining transgenic poppy plants could be obtained.

Accordingly, the invention relates to the use of a buffer to prevent, reduce the rate of, or delay the rise in the pH around the poppy cells during transformation as a defined in claims 1 and 2 as a amended, and/or during culturing of the transformed cells as recited in claim 3 as a amended to generate a transgenic alkaloid producing poppy plant, rather than the determination of a transgenic alkaloid producing poppy plant optimal transformation and culture conditions for each poppy species. Further to this and as stated at page 7, lines 1 to 8, methods of the invention are based on the use of conventional methods of plant transformation and regeneration techniques but which include the additional step of stabilising pH of the medium. That is, the invention *adds* to conventionally known methods of plant transformation and regeneration of alkaloid poppy producing plants and allows for

the use of known culture conditions. Further to this, and as particularly noted by the Applicants at page 7, lines 14 and 15 of the instant specification, the occurrence of a rapid rise in pH during culturing of poppy cells may be readily ascertained by those skilled in the art. Once the presence of the rise in pH is known, it is then a matter of routine trial and experimentation to supplement the culture medium used with a suitable buffer at an appropriate concentration to prevent, reduce the rate of, or delay the rise in the pH and thereby implement the method of the invention. While it is of course desirable to optimise transformation and culture conditions as the Examiner has suggested, this is not the crux of the invention. Rather, the invention relates to maintaining pH relatively stable and in particular, avoiding significant rises in pH in the culture of poppy cells. Of course, if the selection of a particular buffer is likely to impact on other components of the culture medium, the skilled addressee knowing the make up of the buffer, can modify the buffer on the basis of basic principles to account for any such impact on other components in the medium. Having recognised a problem of conventional transformation and transgenic plant generation techniques, the Applicant's have provided a solution which can be readily implemented and incorporated into conventionally known methods to thereby increase the probability of obtaining a favourable outcome, that is, obtaining a greater probability of success in regenerating poppy plants and recovering transgenic poppy plants. Given the above and the fact that the specification has provided a concrete example of the application of the invention, it is respectfully submitted the skilled addressee would be able to readily carry out the invention without undue trial and experimentation on the basis of the teachings provided in the instant specification.

Claims 1 to 3, 7 to 13, 24, 28 and 29 have been rejected under 35 U.S.C. 102(b) as being anticipated by De Block 1990 (Plant Physiology 93:1110-1116). De Block relates to a method of producing a transgenic poplar plant comprising transforming a plant seedling stem explant with a heterologous gene, culturing callus and regenerating a transgenic plant. De Block does not teach or suggest transformation of alkaloid producing poppy plant material with exogenous nucleic acid or regenerating a transgenic alkaloid producing poppy plant from such transformed plant material.

The Examiner has also cited Yoshimatsu et al 1996, (pages 243-252 in Biotechnology in Agriculture & Forestry Vol. 38, Plant Protoplants and Genetic Engineering VII, Ed. Y.P.S. Bajaj, Springer-Verlag, Berlin) in support of a rejection under 35 U.S.C. 102(b).

Yoshimatsu discusses the induction of transformed cultures of *P.somniferum* and their ability to produce morphinan alkaloids. More particularly, Yoshimatsu discloses the

transformation of hypocotyl segments using *Agrobacterium rhizogenes MAFF* 03-01724 harbouring a micimopine-type Ri plasmid. Interesting, the disclosure states that a total of three clones of transformed colli were established "*although* the infection rate was below 20%". The transformation of the hypocotyl material was performed in half-strength MS liquid medium while calli were subsequently cultured on HF MS solid medium as set out in the protocols disclosed at pages 249 to 250 of the citation.

The claims of the instant application as amended require that transformation of alkaloid producing poppy plant material and/or the culturing of the transformed plant material occur *in the presence of a buffering agent that prevents, reduces the rate of, or delays the rise in pH of the plant material or culture medium* from a desired pH level. The Examiner has stated that he has interpreted the limitation of "a buffering agent" broadly in view of pending claim 11 which allowed for the possibility of the buffering agent being "a modified ammonium and nitrate ions content in a predetermined ratio". Applicants have now amended claim 11 to recite the buffering agent as being selected from the group consisting of MES buffer, ADA buffer, BIS-TRIS buffer, and a *buffer* having an ammonium and nitrate ions content in a predetermined ratio.

The Applicants submit that half-strength MS and MS solid medium are conventional *unbuffered* culture medium that have no buffering agents added to them for resisting changes in pH to any significant degree. Accordingly, it is submitted Yoshimatsu does not teach or suggest the use of a buffering agent during the transformation of alkaloid producing poppy plant material with exogenous nucleic acid, nor culturing such transformed plant material to provide a transgenic plant in the presence of a buffering agent as now claimed. Indeed, it is submitted Yoshimatsu simply teaches the use of conventional culture medium and does not contemplate the effect of pH rise on transformation and regeneration of transformed plant material whatsoever. Further to this, Applicants submit that a buffer is one which is adapted for the purpose of resisting significant change in pH by the addition of buffering agent(s) for this purpose. Accordingly, a person skilled in the art would not consider the medium utilised in Yoshimatsu as being a buffer in the context of the present invention, or be lead to the present invention by the disclosures provide by Yoshimatsu. Further, given Yoshimatsu is entirely silent on buffering culture medium for the transformation of plant material and/or generation of transgenic plants from the transformed plant material, the citation provides no motivation whatsoever a person skilled in the art to provide the instant invention.

The Examiner has rejected claims 24 to 29 under 35 U.S.C. 102(b) as anticipated by, or in the alternative, under 35 U.S.C. 103(a) as obvious over Yoshimatsu.

Applicants have cancelled claim 25. Accordingly claims 24 and 26 to 29 remain. These claims have been limited to transgenic alkaloid producing poppy plants produced by the method of the invention or to the transformed plant material prepared in the methods and which is used to generate the transgenic alkaloid producing poppy plants. Yoshimatsu does not teach or suggest transforming the poppy plant material in the presence of a buffering agent as now claimed. Accordingly, the transgenic poppy plants and transformed plant material of the instant invention involve the use of a process that is not disclosed in Yoshimatsu.

Claims 14, 15 and 20 have been rejected under 35 U.S.C. 103(a) as being unpatentable over De Block in view of Bidney (US Patent 5, 932,782). Claims 14 and 15 are ultimately appended to claim 2 and so incorporate all of the limitations recited in that claim. De Block does not teach or suggest the methods of the invention and the Applicant's comments above in relation to the disclosures provided by De Block apply equally here. Further, it is submitted Bidney teaches a method of producing a transgenic plant using *Agrobacterium* adhered to microprojectiles that are bombarded at plant cells in order to achieve transformation. Bidney does not teach or suggest transforming alkaloid producing poppy plant material and/or regenerating a transgenic alkaloid producing poppy plant from such material in the presence of a buffering agent to prevent, reduce the rate of or delay a rise in pH to thereby enhance transformation and/or generation of a transgenic poppy plant as now claimed. Accordingly, even by combining the disclosures of De Block and Bidney together, one does not arrive at the instant invention. Applicants have also deleted claim 20 and it is submitted that this together with the amendments made to claims 1 to 3, this rejection has been rendered moot.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page(s) is/are captioned "Version with markings to show changes made".

Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

Respectfully submitted for,
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Page 6. lines 15 to 19 are amended as follows:

[Figure 4 Effects] Figures 4A and 4B Show effects of various NO_3^-/NH_4^+ ratios in culture medium on type I and type II callus weight.

[Figure 5 Effects] Figures 5A to 5C Show effects of various buffering agents on pH of the callus growth medium.

Figure 6 [Effects] Shows effects of various MES buffer concentrations on pH of the callus growth medium.

The paragraph of Page 6, lines 20 and 21 (amended) as follows:

Figure 7 Shows results of a PAT (phosphinothriecin acetyl transferase) Assay. The arrow indicates the radioactive acetylated PPT band resulting from PAT enzyme activity. 1 and 9: A transgenic tobacco extract as a positive control. 2 and 10: A non-transgenic control poppy. 3-8: Various primary transgenic poppy plant extracts, from plants transformed with the pTAB101 binary vector.

The paragraph of Page 6, line 25 is amended as follows:

Figure 8 Shows a w[W]estern blot of seed from transgenic line 45-25, transformed with pBSF16. SSA standards are various amounts of sunflower seed albumin.
C. is control non-transgenic seed extract. T, is transgenic seed extract. The signals results from specific binding of an antiserum to the sunflower seed albumin protein.

Page 8, lines 1 to 4 are amended as follows:

The preferred exogenous genetic material used in transformation is the binary vector TAB101 containing 35S 5′:*pat*:35S 3′ (see Fig. 1).

Another preferred exogenous genetic material is the binary vector BSF16 (see Fig. 2).

A further preferred vector is pPOP5 (see Fig. 3) which has two genes in the T-DNA: the *pat*

In the Claims:

1. (amended) A method of producing a transgenic alkaloid producing poppy plant comprising the steps of:

1) introducing exogenous [genetic material] nucleic acid for conferring a selected property on the transgenic plant into plant material in the presence of a buffering agent which prevents, reduces the rate of, or delays [the] a rise in pH, of [culture medium or] the plant material or culture medium for culturing of the plant material, from a desired pH level;

2) culturing [said] the plant material in the presence of [a] the buffering agent; [which prevents, reduces or delays the rate of rise in pH of the culture medium or plant material;] and

3) [regenerating a] generating the transgenic plant from [said] the plant material.

Please amend claim 2 as follows:

2. (amended) A method of transforming [a] an alkaloid producing poppy plant to provide a transgenic plant comprising the step of introducing exogenous [genetic material] nucleic acid for conferring a selected property on the transgenic plant into plant material of the poppy plant in the presence of a buffering agent which prevents, reduces the rate of, or delays [the] a rise in pH of [culture medium or] the plant material, or culture medium for culturing of the plant material from a desired pH level.

Please amend claim 3 as follows:

3. (amended) A method of producing a transgenic alkaloid producing poppy plant from plant material harbouring exogenous [genetic material] nucleic acid for conferring a selected property on the transgenic plant, comprising the steps of:

1) culturing [said] the plant material in culture medium in the presence of a buffering agent which prevents, reduces the rate of, or delays [the] a rise in pH of the culture medium or the plant material; and

2) [regenerating] generating [a] the transgenic plant from the plant material.

Please amend claim 4 as follows:

4. (twice amended) The [A] method according to claim 2 wherein the transgenic plant is an alkaloid producing [poppy plant] *Eschscholtzia* species.

Please amend claim 5 as follows:

5. (amended) The [A] method according to claim [4] 2 wherein the transgenic plant is [selected from the] a *Papaver* species [or *Eschscholtzia* species].

Please amend claim 6 as follows:

6. (amended) The [A] method according to claim 5 wherein the [plant] *Papaver* species is *Papaver somniferum*.

Please amend claim 7 as follows:

7. (twice amended) The [A] method according to claim 2 wherein the plant material is derived from seeds, imbibed seeds or seedling parts of the plant.

Please amend claim 8 as follows:

8. (twice amended) The [A] method according to claim 2 wherein the plant material is selected from the group [comprising] consisting of seed explant, seedling explant, type I callus, type II callus, somatic embryogenic callus, any culture which gives rise to somatic embryos [or], and any culture which gives rise to shoots and plant tissues [such as leaves, stems, roots or flowers].

Please amend claim 9 as follows:

9. (twice amended) The [A] method according to claim 2 wherein the rise in [the] pH is prevented or delayed.

Please amend claim 10 as follows:

10. (twice amended) The [A] method according to claim 2 wherein the pH is maintained between pH 5.5 and 6.5.

Please amend claim 11 as follows:

11. (twice amended) The [A] method according to claim 2 wherein the buffering agent is selected from the group consisting of 2-[N-morpholino]ethane sulfonic acid buffer (MES), N-[2-acetamido]-2-iminodiacetic acid buffer (ADA) and bis[2-hydroxyethyl]iminotris-[hydroxymethyl]methane buffer (BIS-TRIS) [or a modified], and a buffer having an ammonium and nitrate ions content in a predetermined ratio.

Please amend claim 12 as follows:

12. (twice amended) The [A] method according to claim 2 wherein the exogenous [genetic material] nucleic acid is introduced into plant cells by a plant transformation agent.

Please amend claim 13 as follows:

13. (amended) The [A] method according to claim 12 wherein the transformation agent is *Agrobacterium tumefaciens*.

Please amend claim 14 as follows:

14. (twice amended) The [A] method according to claim 2 wherein the exogenous [genetic material] nucleic acid is introduced using a mechanical method.

Please amend claim 15 as follows:

15. (amended) The [A] method according to claim 14 wherein the mechanical method is microparticle bombardment.

Please amend claim 16 as follows:

16. (twice amended) The [A] method according to claim 4 or claim 5 wherein the exogenous [genetic material] nucleic acid encodes a mRNA or protein that confers on the [transgenicplant] transgenic plant a property selected from the group [comprising] consisting of:

increased alkaloid yield relative to the native alkaloid producing plant, increased herbicide resistance relative to the native alkaloid producing plant, increased soil acidity tolerance relative to the native alkaloid producing plant, increased disease resistance relative to the native alkaloid producing plant, increased inset resistance relative to the native alkaloid producing plant, increased growth rate relative to the native alkaloid producing plant, improved flowering properties relative to the native alkaloid producing plant, increased frost tolerance relative to the native alkaloid producing plant and altered alkaloid proportions relative to the native alkaloid producing plant.

Please amend claim 17 as follows:

17. (twice amended) The [A] method according to claim 4 or claim 5 wherein the exogenous [genetic material] nucleic acid encodes a mRNA or protein that confers on the transgenic

poppy the property of altered alkaloid proportions relative to the native alkaloid producing plant.

Please amend claim 18 as follows:

18. (twice amended) The [A] method according to claim 4 or claim 5 wherein the exogenous [genetic material] nucleic acid encodes a mRNA or protein that confers on the transgenic poppy the property of herbicide resistance.

Please amend claim 19 as follows:

19. (amended) The [A] method according to claim 18 wherein the herbicide resistance is selected from the group consisting of Basta herbicide resistance, glyphosate resistance, bromoxynil resistance and sulfonylurea resistance.

Please amend claim 24 as follows:

24. (twice amended) A transgenic plant prepared by the method of claim 1 or claim 2.

Please amend claim 26 as follows:

26. (amended) The [A] transgenic plant according to claim [25] 24 wherein the plant is selected from the group consisting of [a] *Papaver* species [or] and *Eschscholtzia* species.

Please amend claim 27 as follows:

27. (amended) The [A] transgenic plant according to claim 26 wherein the *Papaver* species is *Papaver somniferum*.

Please amend claim 28 as follows:

28.(twice amended) [Plant material when prepared by a] The transformed plant material produced by the method according to claim 1 or claim 2.

Please amend claim 29 as follows:

29. (amended) The transformed plant [Plant] material according to claim 28, selected from the group [comprising] consisting of seed explant, seedling explant, type I callus, type II callus and somatic embryogenic callus.

Please cancel claims 20, 21, 22, 23 and 25.